CLASSIFICATION OF PATHOGENIC STREPTOCOCCI BY FERMENTATION REACTIONS *

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The question of the unity or multiplicity of the streptococci, which arose soon after the first description of the organisms, is still unsettled. The recent work of Rosenow throws new light on the whole subject and indicates that if distinct species exist, rapid mutations from one to another may take place. The fact remains, however, that, as we meet them in nature, streptococci differ widely in their microscopic appearance, their manner of growth, their metabolic activities, and especially in their pathogenic properties. We meet with strains which grow on healthy or even abraded mucous membranes without any tendency to invade or to produce a reaction in the host. We meet with others which set up generalized infections of the most severe type, and with all gradations between these two extremes. Whether we regard these as distinct species or as variants of one species, it is of obvious importance to determine with which type we have to deal in any given case. Numerous tests have been devised to this end, but there seems to be no general agreement as to their value. Virulence for small animals does not seem to run parallel with that for human beings, and the classification according to hemolytic power, the method most generally used, is in some ways unsatisfactory as we shall show.

The application of the carbohydrate-splitting powers to classification, which proved of such value in the typhoid-colon-dysentery group of bacilli, has been attempted in the case of the streptococci in a desultory way by many observers, but very thoroughly by Gordon, Houston and Andrewes and Horder. The work of these English investigators has received scant attention, due perhaps to the complexity of Gordon's results. We were attracted to the subject by the reports of Winslow and Palmer[1] and others in this country who attacked this problem by making quantitative determinations of the acid produced in various sugars. They obtained results which differed somewhat from those of the English observers, and attributed their differences to the greater accuracy of their own methods.

The first extensive work on the fermentative reactions of streptococci was that of Gordon.[2] He first selected 10 strains of streptococci from various sources

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1. *Jour. Infect. Dis.*, 1909, 10, p. 288.
2. *Rep. Med. Off. Loc. Gov. Bd.*, 1903, 33, p. 388.

and tested the actions of these on 14 carbohydrates, 13 glucosides and 6 polyatomic alcohols. To many of these tests all strains reacted identically, and he selected 7 substances as of the greatest differential value, namely, lactose, saccharose, raffinose, inulin, salicin, coniferin and mannite. He also studied coagulation of milk, and the reduction of neutral red broth in anaerobic culture. These 9 reactions, since known as "Gordon tests," he then applied to 300 strains of streptococci isolated from 22 samples of saliva and found 48 groups in his 300 strains, which he indicated by letter and number (2a, 2b, etc.). There were, however, only 10 types of which 10 or more specimens were found. He also examined a few pathogenic strains. The characteristics of each strain were constant on repeated tests, except that a single animal passage in two instances altered the reaction (once to salicin and once to neutral red). Otherwise they remained constant.

Shortly afterward, Houston[3] applied all the Gordon tests except that of coniferin to 300 strains of streptococci isolated from 20 human stools. He described 40 types differentiated by these reactions, but was able to bring about 60 per cent of the strains under 10 heads. The following year the same investigator[4] reported tests made on 100 strains isolated from 10 specimens of cow dung. These showed, in general, some differences from the human type. For example: many of these fermented raffinose, whereas raffinose was attacked by very few of his human strains.

TABLE 1.

CLASSIFICATION OF STREPTOCOCCI BY ANDREWES AND HORDER

	Milk Clot	Neutral Red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Coniferin	Mannite	Growth on Gelatin 20° C.	Morphology	Pathogenicity to Mice
A Str. equinus	±	−	+	+	−	−	−	±	−	−	medius	−
B Str. mitis	+	+	+	+	±	−	±	±	−	+	brevis	+
C Str. pyogenes	+	±	+	+	±	−	±	±	−	+	longus	+
D Str. salivarius	+	+	+	+	+	−	+	−	−	−	brevis	−
E Str. anginosus	+	+	+	+	+	−	+	−	−	+	longus	+
F Str. fecalis	+	+	+	+	+	±	±	±	+	+	brevis	+
G Pneumococcus	±	+	+	+	+	−	±	±	−	+	brevis	+

Neither Gordon nor Houston suggested any satisfactory grouping of their numerous types, and the first attempt to clear the situation was made by Andrewes and Horder.[5] They studied 288 strains from human lesions. Unlike Gordon and Houston's organisms, each strain was obtained from a distinct source (except in a few instances where more than one fermentative type was isolated from a sore throat or similar lesions, and so reduplication was avoided. The type of infection, caused by the organisms, gave them a clue to the significance of the metabolic tests. By systematic tabulation of their results and of those of previous workers, they were able to establish 7 types about which they grouped all the forms encountered. In addition to the Gordon tests they claimed differential value for the type of chain formation (longus, brevis and medius), the ability to grow on gelatin at 20 C., and the pathogenicity to mice. Their results are summarized in Table 1.

3. *Ibid.*, p. 472.
4. *Ibid.*, 1904, 34, p. 358.
5. *Lancet*, 1906, 2, p. 708, 775 and 852.

As these types will be referred to frequently, we may be permitted to describe them briefly. The authors insist that no sharp boundaries can be drawn between the groups, and that any tabulation of results must be tentative. As we will see later their grouping of aberrant types under the various heads may justly be questioned.

A. The streptococcus equinus, a saprophyte growing in the intestine of herbivora, consequently found in city air and dust; found occasionally in human feces and saliva; never pathogenic; characterized by inability to ferment lactose or to acidify milk. Most forms ferment saccharose and the glucosides, and a few ferment raffinose or reduce neutral red.

B. The streptococcus mitis, a short-chained form; almost never pathogenic; never found in suppurative processes; found frequently in saliva and human feces; grows well at 20 C.; does not coagulate milk; frequently reduces neutral red; ferments lactose and saccharose, frequently salicin and coniferin, and occasionally raffinose or inulin.

C. The streptococcus pyogenes, usually found in suppurative lesions and in septicemias. It is the most important pathogenic type and "in its fully developed form seems not to occur as a human saprophyte, though some of its variants have been met with in the saliva and feces. . . . It is a long-chained form usually growing in woolly masses at the bottom of a clear broth, occasionally the broth is more turbid and the chains only of medium length." It grows well at 20 C.; is actively hemolytic; does not clot milk or reduce neutral red; is pathogenic to mice and rabbits; ferments saccharose, lactose, and usually salicin. Any one of these reactions may be "suppressed," or other reactions may be added, and some variants are found which ferment coniferin or mannite, or liquefy gelatin, and a few variants ferment raffinose and inulin.

Andrewes and Horder regard this as a parasitic form which has evolved from Type B. It is not to be differentiated from the streptococcus mitis culturally, but only by the fact that it forms long chains, and is usually associated with suppurative processes.

D. The streptococcus salivarius, a short-chain form which clots milk and usually clouds broth, is the most common type found in the mouth; but also found often in human feces. It usually reduces neutral red; often fails to grow at 20 C.; ferments saccharose and lactose, usually raffinose, sometimes salicin or coniferin, and rarely inulin; is usually non-pathogenic, but otherwise closely related to the pneumococcus.

E. The streptococcus anginosus, found most frequently in the angina of scarlatina; seems to be a pathogenic form of the "salivarius"; its fermentative reactions are the same as in Type D, from which it differs in forming long chains, in failing to cloud broth, and in being actively hemolytic. In these respects it resembles Type C.

F. The streptococcus fecalis, found most often in the feces; practically never in the mouth, but is sometimes associated with inflammations, especially with cystitis; usually forms short chains and clouds broth, in which it produces H_2S; ferments mannite always, saccharose, lactose, salicin and coniferin usually, and occasionally raffinose or inulin; it usually clots milk and reduces neutral red, and occasionally liquefies gelatin; it is not hemolytic.

G. Pneumococcus. Type characterized by the formation of a capsule under favorable conditions; the fermentative reactions are very variable, but it usually ferments saccharose, lactose and raffinose, frequently induces coagulation in milk, and ferments inulin; it rarely ferments salicin, coniferin or mannite, or reduces neutral red.

The analysis of the pathologic conditions, in which these various types are met, is of great interest, but for details one must refer to the original article.

Later, Gordon reviewed the work of Andrewes and Horder and accepted their classification;[6] he also reported the results of the examination of 155 strains isolated from the throat in 50 cases of scarlatina.[7] Organisms of the pyogenes type predominated, and were found in 38 of the 50 cases examined.

The classification given by Andrewes and Horder has much to recommend it, but the numerous ± symbols in their table indicate that the classification lacks rigidity, and that a given strain is placed under one or under another head according to individual judgment.

Their results have not attained general acceptance. Walker[8] found the fermentation reactions inconstant. According to his experiments, tests made on one and the same strain at intervals of weeks or months may give very different reactions on the Gordon media. By growing a strain in broth containing a sugar which it did not ferment for a number of generations, he was able in many instances to confer on it the ability to ferment this particular sugar. He was able to do this with a considerable number of strains, but not with all. On the other hand, after growing for several generations on milk, several strains lost their ability to coagulate milk. These results are at variance with those of other workers.

Buerger[9] studied the fermentation of 34 strains, mostly pathogenic, but was unable to confirm Andrewes and Horder's results.

An attempt to classify streptococci by fermentative tests, quite independent of the work of the English investigators, was made by Salomon.[10] This investigator examined the reaction of 78 organisms, streptococci, pneumococci and specimens of the streptococcus mucosus. He used 10 per cent. solutions of the test substances colored with litmus. Many of the reactions were of no differential value, but he found that glycerin, mannite, raffinose, arabinose and soluble starch were fermented by some strains and not by others. On the basis of these 5 reactions, he divided the pathogenic types into 4 groups:

"A. Streptococcus pyogenes.
  I. Streptococcus pyogenes fermenting starch only.
  II. Blood culture strains fermenting glycerin and mannite.
B. Streptococcus mucosus.
  I. Fermenting glycerin, mannite and arabinose.
  II. Not fermenting."

He also studied the fermentation of a number of strains in these 5 substances quantitatively by titration with phenolphthalein, but obtained no results of differential value.

Winslow and Palmer[11] based their work on the assumption that the change in color of litmus broth is not an accurate indication of the fermentative properties of bacteria. Streptococci form a slight amount of acid in most media, and the amount formed in those containing a fermentable sugar varies greatly with different types of these organisms. Their method was to plant a large series of strains in broth containing a certain carbohydrate. The amount of acid formed

6. *Jour. Path. and Bac.*, 1911, 15, p. 323.
7. *Rep. Med. Off. Loc. Gov. Bd.*, 1910, 40, p. 302.
8. *Proc. Roy. Med. and Chir. Soc., London,* 1910-11, 83 B, 541.
9. *Jour. Exper. Med.*, 1907, 9, p. 428.
10. *Centralbl. f. Bakteriol.*, 1908, 47, p. 1.
11. *Jour. Infect. Dis.*, 1910, 7, p. 1.

in 3 days was determined by titration, using phenolphthalein as an indicator—the acidity of uninoculated broth being subtracted from the result. The number of strains causing a given degree of acidity were then arranged in a frequency table and a curve plotted showing how many strains produced from 0 to 0.5 per cent. normal acidity; how many produced 0.5 to 1.0 per cent.; how many produced 1.0 per cent. to 1.5 per cent, etc. These curves as a rule showed two modes, one about zero or below, and the other above 1.0. Between these the curves dropped to a low point about 0.5 per cent., and this was taken as the dividing line, i. e., strains which produced 0.5 acidity or more were considered as fermenting; those which produced less, as non-fermenting. They used 3 of the Gordon media; lactose, raffinose and mannite broth, and also dextrose broth, and tested 116 strains of streptococci from human feces, 100 from horse dung, and 100 from cow dung. They grouped all of these, except a few abberrant types, in 9 classes. So far as could be determined from these tests, most of the strains correspond to some of Andrewes and Horder's types. Winslow and Palmer, however, subdivided these groups by the dextrose test (Table 2).

TABLE 2.

CLASSIFICATION OF FECAL STREPTOCOCCI (WINSLOW & PALMER.)

Carbohydrate Fermented	Name of Type (Andrewes and Horder)	Streptococci Found in		
		Man, Percentage	Horse, Percentage	Cow Percentage
None	Str. equinus	9	15	18
Dextrose alone	Str. equinus	23	73	27
Dextrose and lactose	Str. mitis	31	5	21
Lactose and raffinose	Str. salivarius	0	0	12
Dextrose, lactose and raffinose	Str. salivarius	5	0	9
Dextrose, lactose and mannit	Str. fecaiis	23	0	2

The strains of the "equinus" type from man differed from those from the cow and horse in forming nearly twice as much acid in dextrose, and, in general, streptococci of human origin formed more acid in dextrose than other strains. The frequency curves for the human strains showed the mode at about 3.8 per cent., as against 1.8 per cent. for the equine, and 2.3 per cent. for the bovine strains.

The significance of these results is considerably lessened by the fact that like Gordon and Houston, Winslow and Palmer tested many "strains" from one source. Their 116, 100 and 88 strains were isolated from 15, 12 and 22 samples of human, equine, and bovine excreta, respectively. As many as 23 colonies were fished from a single specimen of feces and each was regarded as a separate strain, although many of them fermented in a practically identical manner. For example, the 23 per cent. of the streptococcus equinus from human feces was found in 5 of the specimens examined, and the 73 per cent. from the equine was found in 10 specimens.

Stowell and Hilliard[12] studied 70 strains from inflamed throats, from normal throats, and from milk. They titrated the acid formed in dextrose, lactose, maltose, raffinose, saccharose, and mannite, both at 37 C. and at 20 C. The results

12. *Amer. Jour. Dis. Child.*, 1912, 3, p. 287.

led to no definite classification. They met with surprisingly few raffinose fermenters, considering the source of their organisms, and with none which fermented mannite.

Broadhurst[13] studied quantitatively the fermentations of 100 strains, isolated from 100 samples of cow's milk, on 6 carbohydrates—lactose, saccharose, salicin, raffinose, mannite, and inulin. She obtained much higher readings than Winslow and Palmer and found the "intermodal point" separating fermenters and non-fermenters to be about 1.5 per cent. She constructed frequency polygons from her own figures and from those published by Winslow and Palmer. Studied in this way, the milk streptococci resembled the human strains of Winslow and Palmer more than the bovine or equine. Qualitatively, the results were difficult to summarize. The strains fell into 19 groups, the largest containing 15 members. Only a few corresponded to the Andrewes and Horder types; but the majority may be described as atypical members of the following classes: the streptococcus fecalis, 27; the streptococcus anginosus, 8; the streptococcus pyogenes, 38; the streptococcus equinus, 23.

Following this, Winslow[14] summarized all the work with quantitative titrations published. He concluded: "All these more or less preliminary results suggest that the biometric study of fermentative powers may not only throw light on the systematic relationships of the cocci, but may yield results of practical sanitary importance."

Stowell, Hilliard and Schlesinger,[15] in a second paper, reported further studies of streptococci from milk and human throats. They arrived at no definite classification, but concluded that milk strains were characterized by high acid production, by ability to grow at room temperature, and by inability to ferment complex substances; that throat strains, on the other hand, usually fermented some more complex substances than the disaccharids at 37 C., but failed to do so at 20 C.

A recent paper by Fuller and Armstrong[16] recorded a study of fecal streptococci which practically confirmed the results of Winslow and Palmer. They tested 349 strains on 7 media, using saccharin, salicin and inulin in addition to the media employed by Winslow and Palmer, but found the additional tests of little value in grouping the various strains.

In the second paper, Broadhurst[17] compared the amount of acid produced in meat extract broth with that produced in meat infusion broth. She tested a number of strains against various carbohydrates, and found with few exceptions a much larger amount of acid in the infusion media than in the extract, when the sugar was fermented. One would infer that the infusion media giving better growth accentuate fermentative differences and are more favorable for this purpose.

We may summarize the facts gleaned from the literature as follows: The classification of streptococci according to fermentation reactions alone has led to the establishment of a bewildering number of types. Andrews and Horder grouped all these types under six heads to which they gave specific names. They showed a definite relation between their grouping by cultural tests and the pathogenicity and source of the organisms.

13. *Jour. Infect. Dis.*, 1912, 10, p. 272.
14. *Ibid.*, p. 285.
15. *Ibid.*, 1913, 12, p. 144.
16. *Ibid.*, 13, p. 442.
17. *Ibid.*, p. 404.

This classification has been attacked on several grounds: (1) Walker asserted that fermentation reactions were not constant in any strain, but vary from time to time; (2) Buerger found that such a grouping does not correspond to the type of pathogenicity of the strain; (3) Winslow and others in this country maintained that the quantitative determination of the acid produced was essential to an accurate study of fermentation. However, after very extensive work on saprophytic types they have arrived at no very definite results; (4) Salomon proposed a quite different grouping of the streptococci based on fermentation tests not used by Andrewes and Horder.

PLAN OF WORK

Three important questions remain unanswered. Is the ability to ferment carbohydrates and allied substances a constant characteristic of strains of streptococci as it is of members of the colon-typhoid-dysentery group of bacilli, and consequently a sound basis for classification? Is the amount of acid produced in fermentation constant, and of value? Does any constant relationship exist between the fermentation reactions, qualitative or quantitative, and the pathogenicity or source of the organism?

As much of the qualitative and practically all of the quantitative work had been done on saprophytic strains which are of little importance in medical bacteriology, we determined to use strains known to be pathogenic to man. We collected streptococci from as many types of lesions as possible.[18] They were tested as soon as possible after isolation, most of them within two weeks, but some had been on artificial media for longer periods. The Neufeld bile-test was made on all specimens and only those strains which were bile insoluble were included in our studies. They were grouped as follows according to source: (1) Definitely pathogenic. Streptococcus strains obtained from blood of infected patients, or from closed suppurative processes; (2) probably pathogenic. Strains obtained in mixed culture from inflammatory exudates, for example, from crypts of inflamed tonsils; (3) saprophytic. Streptococcus strains from normal throats, from milk and from human stools. The fermentation reactions of such saprophytic races have been thoroughly studied by others as indicated above; but as the methods followed have been different in each case

18. Most of these were from patients in St. Luke's Hospital, but some were obtained through the courtesy of Dr. W. P. St. Lawrence, Dr. H. L. Celler, Dr. A. R. Dochez and Dr. D. S. D. Jessup.

it seemed advisable that we should include a few members of this group to determine whether the differences in method had any marked effect on the results. The streptococci in the first group were found in pure culture; those in the last two groups were carefully plated before making the tests. Our work on these micro-organisms has included studies of morphology, hemolysis, milk coagulation, reduction of neutral red, fermentation of carbohydrates and allied substances.

Our conclusions are based on the observation of these tests in 105 strains of streptococci. We will take up these various tests in order, discussing the methods used and the results obtained. We have also made fermentation tests on a number of strains of pneumococcus and of pneumococcus (or streptococcus) mucosus for comparison with the streptococci. These are included in Table 5 under separate heads.

I. MORPHOLOGY

We used only strains which showed chains of six or more members in broth, but accepted some in which diplococcus forms predominated, though occasional chains were present. We included in the series two strains which showed capsules when first isolated but which otherwise resembled streptococci. Our records (Table 5) are based on smears of sediment of twenty-four-hour dextrose broth cultures. Those forming chains of less than ten members are classed as brevis (B), those of ten to fifteen members as medius (M) and those of over fifteen members as longus (L). Three strains which formed very long chains covering several fields are designated as longior (LL).

The early attempts at streptococcus differentiation were based on morphology. Andrewes and Horder used it as a critical test and in their work the streptococcus mitis was differentiated from the streptococcus pyogenes and streptococcus salivarius from streptococcus anginosus chiefly by the length of the chains formed. We found, as have most observers, that the type of chain formation and the size of the individual cocci depend to a large extent on the medium and may vary considerably in a few generations. Long chains were often found in the sediment of a broth culture when pairs were present in the supernatant fluid. Moreover in most smears both long and short chains are found, so that it is difficult to make the readings objective. We found that most of the virulent pyogenic strains formed long chains and that most of those found in mild infections of the mucous membranes formed short chains. There were many exceptions, however.

We met with one morphological type which was fairly distinct. It grew in long chains of several hundred members. The dextrose broth cultures were perfectly clear with a massive gelatinous sediment which was difficult to break up. This type occurs in the mouth and is only slightly pathogenic. We found three typical examples. The fermentation reactions were not the same, and consequently they could not be grouped together in our table. Moreover, we found several very long chain formers which approached this type in morphology and cultural appearance, and one strain which formed chains of about twelve when first isolated, but later grew in chains of fifty or more, and formed a heavy gelatinous sediment. Probably, then, these long chain forms are not a distinct variety but a manner of growth which more than one variety may assume. We do not think, therefore, that morphology can be used as a basis for classification of streptococci.

II. HEMOLYSIS

The observation of hemolysis on blood agar plates introduced by Schottmüller is the most widely used means of differentiating streptococci. We examined most of our strains by making surface streaks on human blood agar and observing them from one to three days. Some strains, notably the mannite fermenters, produced no lysis. Others obtained from septicemias produced a wide zone in less than twenty-four hours. Between these two extremes, however, were all gradations. Many of the pneumococci and strains of the "viridans" type from endocarditis produced a narrow zone in two to three days. The hemolysis depended also on the depth of the medium and the amount of blood it contained, for in an unevenly mixed blood plate it was sometimes observable in the thin, but not in the thicker portions. Moreover a few strains which were fished from colonies surrounded by a narrow cleared zone, and which came from throats, did not later show any hemolytic power. It is difficult, then, to draw a line sharply between hemolyzing and non-hemolyzing types. The property is present to some extent in most streptococci and while in general it is more pronounced in the more virulent type, the differences are essentially quantitative and therefore not altogether satisfactory as the basis of classification. We have attempted to indicate the strength of hemolysis by one, two or three plus marks in our table, but the estimations are necessarily inaccurate.

III. MILK COAGULATION

Milk coagulation was determined by observations on litmus milk after five days' incubation. We found that this property was not constant in all strains, for some acquired it while under observation. It was roughly parallel to high acid production in lactose broth though a few strains were found which formed only small amounts of acid but still coagulated milk. In general, the strains which coagulated milk were of low pathogenicity, and vice versa; yet there were many exceptions, that is, Strain 158, obtained from a rapidly fatal septicemia, coagulated milk in twenty-four hours. Andrewes and Horder consider this one of the most significant tests and their differentiation between the streptococcus pyogenes and streptococcus anginosus is based chiefly on this criterion. The fact that it is not constant to a given strain, however, makes it of little value.

We met with three strains which promptly coagulated the milk, and then peptonized it ("P," Table 5). They also liquefied gelatin, and were probably the type described by MacCallum and Hastings as the micrococcus zymogenes.[19] The three strains did not show identical fermentation reactions.

IV. REDUCTION OF NEUTRAL RED

We made this test in 1 per cent. dextrose-neutral-red broth covered with liquid petrolatum. Only five strains reacted. We have not found a description of the technic used by others in making this test, but as our results differed so widely from theirs it seemed unlikely that our method was the same. The tests as done by us were of no assistance in classification.

V. FERMENTATION OF CARBOHYDRATES AND ALLIED SUBSTANCES

As this was the part of the work which chiefly interested us, we will describe our methods in some detail and then discuss our results: (1) as to the qualitative study of the presence or absence of fermentation in the various test substances; and (2) as to the quantitative study of the amount of acid produced.

METHODS

The media used in our first tests were made from veal infusion broth containing 1 per cent. peptone (Witte) and 0.5 per cent. sodium chlorid adjusted to about 0.3 per cent. acidity. To this was added 1 per cent. of the fermentable sub-

19. *Jour. Exper. Med.*, 1899, 4, 521.

stance to be tested. As many of our strains grew feebly in this medium, we later adopted 2 per cent. peptone broth which seemed more favorable. The tubes were inoculated at first with a loop-full of growth from a twenty-four-hour agar or ascitic-agar slant—later with a few drops of a twenty-four-hour dextrose broth culture from a capillary pipet. The latter method gave more constant results. The tubes were incubated for twenty-four hours and 5 c.c. of the contents titrated against N/20 normal NaOH, using the first pink of phenolphthalein as the end reaction. The residue was tested by the addition of a few drops of litmus solution. A smear from one or more tubes was examined to determine whether growth had taken place. All the tests were made in duplicate, and when there was any material discrepancy, they were repeated as many times as seemed necessary. A control tube of each lot of every carbohydrate was titrated and the percentage of acidity deducted from that of the inoculated tubes. A second correction was made by inoculating a tube of plain broth (as a rule from the same lot of meat infusion as the sugar broth) and deducting the amount of acid formed from the result. This procedure was adopted to avoid the use of colon free-sugar broth recommended by some investigators, partly because many streptococci grow feebly in colon broth, and partly to exclude possible acid formation from non-carbohydrate constituents. The twenty-four-hour period was chosen because very little acid is formed after this time, as shown in Table 3. Two low fermenting strains and two high fermenting strains were tested in a dextrose broth after incubation for varying periods, and the readings after thirteen hours were about the same as after five days.

TABLE 3

RAPIDITY OF ACID PRODUCTION BY FOUR STRAINS OF STREPTOCOCCI

	13 Hours	29 Hours	53 Hours	73 Hours	120 Hours
Strain 108.....	4.8	5.7	6.1	5.6	6.3
Strain 157.....	5.2	5.7	5.6	4.5	5.4
Strain 9.....	3.6	3.4	3.4	3.7	2.8
Strain 158.....	3.5	3.4	3.4	...	3.6

Only the results obtained from 2 per cent. peptone media were used in plotting frequency curves, as these ran from 0.3 per cent. to 1.2 per cent. higher than those obtained on 1 per cent. peptone. Where both of the duplicate tests were made on this medium, the average of the results was recorded. Where the first test was made on 1 per cent. peptone broth and the results on 2 per cent. agreed qualitatively, the latter figures were used in constructing Table 5. A number of strains tested only on 1 per cent.peptone and starred (*) are included in our table.

The fermentable substances used were the "Gordon test media," with the changes recommended by Stowell and Hilliard, namely; dextrose; lactose, saccharose; salicin, raffinose, mannite and inulin. Later, to compare our results with those of Salomon, glycerin, soluble starch and arabinose were used. The lactose, salicin, raffinose, glycerin and starch used were Merck's. The saccharose was also Merck's "reagent." The mannite was Kahlbaum's; the dextrose, Eimer & Amend's c. p.; the inulin, Eimer & Amend's "Kiliani." Part of the arabinose tests were done with sugar of Kahlbaum's preparation; the others with a preparation obtained from Eimer and Amend. For the litmus test, we used Merck's "reagent" grade in 1 per cent. solution.

QUALITATIVE RESULTS

All tubes in which 0.8 per cent. normal acidity or more was produced were considered positive, and all less than this, negative, for reasons which we will discuss in considering the quantitative results.

Are these results constant in any given strain? From our observations we are unable to assert definitely that they are constant, but what evidence we have obtained points in that direction. We have re-examined twenty-four of our strains at intervals of eight to thirty-four weeks and found the reactions qualitatively unchanged. In other instances we have found discrepancies which are difficult to explain. Not infrequently a reaction was found negative on a single test which later was consistently positive. This we attributed to feebleness or absence of growth in the unfermented tubes. In a few instances, fermentation was obtained on the first test but not on subsequent tests, even though frequently repeated. We can account for these latter cases only by assuming some error, such as a misplaced label or contamination of the tubes. Most of the inconsistencies were in the non-hemolyzing organisms which grew feebly on most of our media. One of the most puzzling examples were the results with Strain 126 on salicin which were as follows: May 31, 1.4; June 26, 0.3; June 27, 0.4; September 16, 1.1; September 16, 2.5; September 18, 2.8.

Strain 125, of the "viridans" type, failed to ferment lactose on the first two tests, and grew feebly. In ascitic fluid-lactose broth it gave a positive reaction and when two plain lactose broth tubes were subsequently inoculated with large amounts of a young culture, it gave positive fermentation in both. These were exceptional cases. The latter may be an instance of acquired fermentative property, but more probably of adaptation to the new environment—the streptococcus fermenting the broth as soon as it was able to grow actively on it. Obviously an organism will not ferment a broth in which it will not grow, and apparently it may also grow feebly in a broth too unfavorable to permit fermentation. The best illustration of the effect of the unsuitability of the medium on fermentation was in the pneumococci. Some strains which repeatedly failed to ferment inulin broth in which they appeared to grow gave typical fermentation in Hiss' inulin serum water. Broadhurst has shown also that more acid is produced in infusion-broth than in extract. The fact that our strains fell into a small number of groups more completely than those of other observers,

we attribute to the use of richer media. Reactions might well be missed in the extract broth or colon sugar-free broth which many have used, as they are poor media for streptococci. We have not used the method of Walker, that of growing a strain for a number of generations on a non-fermentable sugar to produce a positive reaction. We believe that further work is necessary to determine the permanency of the fermentative characteristics. However, in the great majority of instances the results of repeated tests have agreed, and in no instance (except possibly the one cited, Strain 125) could we determine that a consistently negative reaction became consistently positive, or the reverse. Even if it is proven that it is possible to change the reactions of streptococci by artificial methods, this does not necessarily destroy the practical value of the tests. Twort[20] has claimed that by methods similar to those of Walker, mentioned above, he could alter typhoid bacilli so that they would ferment lactose, but, though this be true, it is well established that failure to ferment lactose is characteristic of typhoid bacilli as found in nature.

After we had concluded that these reactions were sufficiently constant to be of value, our next consideration was to determine whether there was any relation between the fermentative powers of a given streptococcus and its pathogenicity or source. We determined to disregard other tests and group them strictly according to their fermentation reactions and then see whether we could correlate such a grouping with our information in regard to their origin. Some of the tests we discarded because, for instance, dextrose was fermented by every strain we tested. Only three showed a positive reaction in glycerin. (Two of these also fermented the hexahydric alcohol mannite.) None of those tested on arabinose fermented it. Starch was fermented by fourteen strains. Seven of these agreed in their other fermentative properties and in their pathogenic properties with the pyogenic type described below, while others did not. Consequently we also discarded this test in defining our groups. On the basis of the remaining tests nearly all the strains examined fell into six groups. On examining the sources from which the cultures in these groups were obtained, we were able to characterize one fermentative group as pathogenic and the others as essentially saprophytic. Members of four of the saprophytic groups were found chiefly in the mouth and

20. Cited from Penfold, *Brit. Med. Jour.*, 1910, 2, 1672.

throat, and those in the remaining group in the intestines. The classification arrived at, was as follows:

A. Pathogenic type:

Group 1, fermenting lactose, saccharose and salicin.

B. Saprophytic types:

a. Salivary types:

Group 2, fermenting lactose, saccharose and raffinose.

Group 3, fermenting lactose, saccharose, salicin and raffinose.

Group 4, fermenting lactose, saccharose, raffinose and inulin.

Group 5, fermenting lactose and saccharose.

b. Fecal type:

Group 6, fermenting lactose, saccharose, salicin and mannite.

Thirteen of the strains examined gave other combinations of fermentation reactions than those given in the above table. Seven of these strains fermented inulin, and for this reason we have classified them under Group 4. Of the remainder, three fermented mannite and were classified under Group 6, and three strains were left unclassified.

Group 1. An examination of Table 5, which gives our series of streptococci grouped in this way, shows that all the highly pathogenic forms, with a few exceptions which we will consider later, fell into Group 1, which we have called the pyogenic group. It includes nine strains from acute septicemia, eleven strains from abscesses and eleven strains from other severe suppurative infections. In this respect our results agree with those of Andrewes and Horder for all the streptococci from abscesses and suppurating wounds which they examined gave these same fermentative reactions. This same type was found in the most severe cases of tonsillitis which we examined, and was recovered from the tonsillar crypts of one of our patients three months after recovery. With this exception, it was not found in normal throats. In a few instances, streptococci of the pyogenic type were found in milder infections of the nose, mouth, throat and bronchi; also once in milk, and once in the air. Most of these pyogenic streptococci grow in long chains, hemolyze blood agar, and do not coagulate milk, but these characteristics are not common to all members of the group.

The remaining types seemed essentially saprophytic. Members of Groups 2, 3 and 4 were found in normal throats. They were also frequently associated with mild infections such as chronic tonsillitis and pyorrhea alveolaris. One of these types (2) occurred in a mild otitis media in a poorly nourished child. The only severe type of infection, in which streptococci belonging to these groups were concerned, was endocarditis. In this disease they were found in many instances. These groups include both long and short chain types. Most of the organisms cause little or no hemolysis on blood agar and the majority coagulate milk.

Group 5 includes streptococci occurring as intestinal saprophytes These races were found in a few severe processes (cystitis, prostatic abscesses, and puerperal sepsis) in which infection from feces seemed probable. They were found also in otitis media (twice) and in infectious endocarditis (twice). Other observers have agreed in considering mannite-fermenting streptococci characteristic inhabitants of the human intestine, and Andrewes and Horder met with them in the same types of infections as did we. These streptococci usually grow in short chains and are not hemolytic. About half of the strains coagulate milk.

Many of the streptococci which we have placed in Groups 2, 3 and 4 answer to the description of the streptococcus salivarius given by Andrewes and Horder and those in Group 5 correspond to the streptococcus fecalis of these investigators. We prefer the grouping given above because it follows rigidly the fermentation tests which are more constant than the characteristics on which they base the differentiation of their types, (viz., morphology and milk coagulation). We were unable to confirm in any respect the findings of Salomon.

So far as we could determine by our observations, a useful differentiation between several distinct types of streptococci can be made by determining qualitatively their ability to ferment lactose, salicin, raffinose, mannite and inulin. Further study is required to determine whether or not tests on saccharose and soluble starch are of value.

QUANTITATIVE RESULTS

Winslow and Palmer claimed for their titration method, (1) that it gave a more accurate verdict than the litmus test in deciding whether or not the test substance in question is fermented; and (2) that a

comparison of the amount of acid formed by various streptococci is a sound basis for classification.

To test the correctness of their first conclusion we have made frequency tables and curves for each fermentable substance used. In each case in which an "intermodal point" between fermenting and non-fermenting groups could be determined, it fell in the class "0.6 per cent. to 1.0 per cent." Consequently we have made our division at 0.8 per cent. and considered the streptococci which produced 0.8 per cent. normal acidity or more as fermenting; others as non-fermenting. On comparing these results with those of the litmus test we found that they were practically identical. The change in the color of litmus from blue to pink was complete in the broth we used at a point 1.2 per cent. to 1.4 per cent. acid to phenolphthalein. In recording our results about 0.7 per cent. was usually subtracted from our readings to correct for the initial acidity of the medium and the acid produced in plain broth. Therefore, in a tube which was distinctly acid to litmus and no more, the corrected reading by titration would be about 0.7 per cent. In short, dropping litmus solution into the tube seemed quite as satisfactory a means for determining whether or not fermentation had taken place as titrating the contents with phenolphthalein.

In considering the value of our quantitative determinations as a basis for classifying the various races, we inquired (1) as to what determines the amount of acid which a streptococcus will produce in a given medium; (2) whether this is a constant property of each strain; and (3) whether this property could be correlated with the pathogenicity, the source, or the qualitative fermentation reactions of the organism.

In regard to the first question it seemed that the production of various degrees of acidity by different streptococci might depend either on a difference in the end products of the fermentation in each case, so that one organism produces more acid (more free H ions) from a decigram of sugar than another, or it might depend on a difference in sensitiveness to the acidity of the medium, that is, one organism might be inhibited in its growth or ferment activity by a lower acidity than another

Stowell and his coworkers take the former view. They say: "The streptococci never carry a fermentation through to the simple elements, carbon dioxid and alcohol, and so we must make our measurements of the amount of fermentative activity that they exert by determining

the amount of acid they split off from the molecule. . . . It is this varying capacity of different bacterial strains to separate uniting bonds existing between atoms and groups of atoms and thus avail themselves of the energy liberated in the process, which we try to measure by acid titration." They present, however, no evidence whatever for the correctness of this rather fanciful hypothesis.

To test the correctness of their theory we planted two low fermenting and two high fermenting strains on broths containing 4 per cent., 2 per cent. and 1 per cent. of dextrose. If the degree of acid produced by each race were dependent only on "the amount of acid they split off from the molecule," it should be proportional to the amount of sugar available. As is shown in Table 4, the acidity was substantially the same regardless of the percentage of sugar.

TABLE 4.

FOUR STRAINS GROWN IN BROTHS OF VARIOUS ACIDITY AND CONTAINING VARIOUS AMOUNTS OF DEXTROSE

Percentage of Dextrose	0	4%	2%	1%	1%	1%	1%
Initial acidity	0.6	0.5	0.5	0.5	2.6	4.3	6.4
Acidity produced in 24 hours							
Strain 108.....	0.6	5.8	5.1	5.3	5.0	4.5	6.6
Strain 157.....	0.6	4.9	4.9	4.8	4.7	3.5	6.5
Strain 9	0.6	2.9	2.9	3.3	2.7	4.5	6.7
Strain 158.....	0.7	2.4	2.6	2.5	2.6	4.3	6.9

From these observations we concluded that the acidity of the broth was limited by some factor which inhibited fermentation—probably the acid itself. To determine this we added varying amounts of sterile normal lactic acid to 1 per cent. dextrose tubes and then inoculated them with the same four strains (Table 4). The final reaction in all the tubes inoculated with each strain was the same (within the limits of error of our method), which shows that fermentation by a given streptococcus ceases when a certain acidity is reached, irrespective of how much acid must be formed to produce this acidity. All we can determine, apparently, by the titration method is the sensitiveness of a particular streptococcus, or its enzyme to acid.

This property might still be of value if it were constant in a given strain. We found, however, that the acidity produced by the same strains in the same medium varied for reasons which we were unable to discover. The results from duplicate tests sometimes checked very closely, especially if made from the same seed tube at the same time; for example:

	Arabinose	Glycerin	Starch	Inulin	Mannit	Raffinose	Salicin	Saccharose	Lactose	Dextrose
Strain 130, Sept. 22.....	4.9	4.5	4.8	4.3	4.3	3.2	3.1	2.4	0.0	0.2
Strain 130, Sept. 22.....	4.7	4.5	4.6	4.6	4.3	3.2	3.1	2.4	0.1	0.0

This was not always the case, however, as is shown in Table 3. Strain 157 which was planted simultaneously on several tubes of 1 per cent. dextrose broth produced only 4.5 per cent. acid in the tube incubated for three days, while those incubated two days and five days titrated 5.6 per cent. and 5.4 per cent. respectively. If duplicate tests were made of the same strain at intervals of several days, rather large variations were apt to occur, for example:

	Dextrose	Lactose	Saccharose	Salicin	Raffinose	Mannit	Inulin	Starch	Glycerin	Arabinose
Strain 112, Aug. 26.....	3.4	4.5	4.7	4.1	0.7	0.7	4.3	0.7	0.7	0.4
Strain 112, Aug. 28.....	3.1	3.8	3.6	2.4	0.6	0.4	3.0	0.4	0.5	0.7

Notwithstanding the fact that the amount of acid produced by a given strain may vary considerably, we grouped the streptococci in our series on the basis of our quantitative studies. We found that in general highly pathogenic strains produced less acid than saprophytic strains, and that the streptococci causing endocarditis produced more acid than the other definitely pathogenic types. There were, however, numerous exceptions to these generalizations, and we were unable to arrive at any satisfactory classification by this method. A comparison of our frequency tables with those for saprophytic streptococci published by other workers also led to no results. In short, we derived no useful information from our titrations that could not have been obtained by the qualitative litmus test.

CONCLUSIONS

Analysis of our experiments seems to justify the following conclusions:

1. The streptococci usually concerned in severe infections in man may be differentiated from the common saprophytic types by fermentation tests.

TABLE 5

RESULTS OF TESTS MADE ON STREPTOCOCCI AND ALLIED ORGANISMS

Serial No.	Source	Pathogenicity	Dextrose	Lactose	Saccharose	Salicin	Raffinose	Mannite	Inulin	Starch	Glycerin	Arabinose	Morphology	Milk	Neutral Red	Hemolysis
	GROUP 1, PYOGENIC TYPE															
4	Blood, sepsis	P	2.2	2.5	2.4	2.8	0.4	—.1	0.2	...	0.3	...	L	0	0	+++
9	Blood, sepsis	P	2.9	2.5	1.2	2.2	—.1	—.1	0.1	0.5	0.1	...	B	0	0	++
10	Blood, sepsis	P	2.1	2.6	2.6	2.1	0.5	0.1	0.4	0.3	0.2	0.3	B	0	0	++
22	Blood, sepsis	P	2.6	2.5	3.0	3.1	0.1	0.0	0.3	0.6	0.1	...	L	0	0	++
61	Blood, sepsis	P	1.0	1.1	1.5	1.3	0.2	0.2	0.0	0.1	0.2	...	L	0	0	+++
145	Blood, sepsis	P	4.2	3.7	3.6	3.2	0.1	—.2	0.4	0.0	0.2	—.1	M	+	0	++
64	Blood, sepsis	P	2.6	2.4	2.7	1.7	0.2	0.0	0.1	0.2	—.2	...	B	0	0	0
102	Blood, sepsis	P	1.2	1.2	1.5	1.0	0.2	0.0	—.1	1.4	—.1	—.6	B	0	0	+++
158	Blood, sepsis	P	2.0	2.2	2.4	2.5	0.1	0.3	0.1	2.2	0.3	0.0	M	+	0	++
76	Blood, pyemia	P	1.1	1.2	1.4	1.3	0.2	0.1	0.1	...	...	...	B	0	0	+
21	Pus, abscess	P	2.5	1.8	1.6	1.1	0.6	0.3	—.1	0.6	0.3	...	B	0	0	++
52	Pus, abscess	P	2.8	2.4	2.4	2.2	0.4	0.2	0.0	0.2	0.2	...	L	0	0	+
72	Pus, abscess	P	2.2	2.9	2.1	1.5	0.4	0.2	0.0	0.4	0.4	—.3	L	0	..	+++
90	Pus, abscess	P	1.3	2.0	2.6	1.3	—.2	0.1	0.2	0.1	—.1	—.5	L	0	0	++
103	Pus, abscess	P	1.7	1.9	2.6	1.6	0.0	—.1	0.2	0.0	0.1	—.4	L	0	0	+++
119	Pus, abscess	P	1.8	2.1	1.6	1.6	0.3	0.0	0.2	0.0	—.2	0.0	B	0	0	++
77	Pus, abscess	P	1.3	1.2	1.1	1.2	0.2	—.1	—.1	...	...	...	B	0	0	+
32	Pus, abscess	P	2.4	3.3	1.2	1.9	0.7	0.5	0.5	0.5	0.6	0.5	L	0	0	+++
70	Pus, abscess	P	1.1	1.0	1.3	1.5	0.3	—.1	0.0	2.2	—.3	0.1	M	0	0	+++
71	Pus, liver abscess	P	1.3	1.5	1.9	1.3	0.3	0.1	0.3	0.1	0.3	—.2	L	0	0	+++
16	Pus, empyema	P	1.8	1.0	1.2	1.7	—.4	—.4	—.4	0.1	—.1	0.0	B	0	0	+
36	Pus, empyema	P	2.3	1.7	2.4	2.7	0.0	0.1	—.3	0.2	—.1	...	L	0	0	+++
34	Pus, pericarditis	P	2.1	2.6	2.4	2.1	0.3	—.5	0.3	—.2	0.0	—.2	L	0	0	++
93	Pus, pericarditis	P	3.9	4.4	2.7	4.0	0.3	0.7	0.0	0.1	0.1	0.0	B	+	0	0
85	Pus, meningitis	P	2.2	1.9	2.3	2.4	0.3	0.2	0.4	0.3	0.1	0.0	M	0	0	0
95	Pus, meningitis	P	1.9	2.4	2.5	2.4	0.4	0.1	0.5	0.5	0.2	—.2	L	0	0	+++
40	Pus, meningitis	P	1.9	2.1	2.0	1.4	0.2	0.2	0.2	2.3	0.1	—.2	L	0	0	++
18	Pus, otitis media	P	1.9	2.0	2.0	1.4	0.3	0.1	—.4	0.2	0.1	...	M	0	0	+
19	Pus, otitis media	P	2.0	2.4	2.4	2.5	—.1	0.2	0.4	0.2	0.3	...	B	+	0	++
28	Pus, folliculitis	P	2.0	2.5	0.8	1.3	0.3	0.1	—.4	—.2	—.1	...	B	+	0	++
78	Gangrene of leg	P	1.4	1.2	1.3	1.0	0.2	0.2	0.1	...	...	...	B	0	0	+++
5	Pus, wound infection	P	1.4	1.5	1.6	1.5	0.3	0.2	0.1	0.6	0.1	0.5	L	0	0	++
124	Blood, endocarditis	P	3.7	3.9	4.1	2.8	0.1	—.1	—.2	—.0	—.1	—.1	..		0	0
126	Blood, endocarditis	P	3.2	3.2	2.9	2.7	0.2	0.0	—.1	0.1	0.1	0.1	..	++	0	0
46	Blood, endocarditis	P	1.6	1.2	1.8	1.2	0.4	0.1	0.5	0.3	0.0	...	B	0	0	....
49	Tonsil, acute tonsillitis	?	2.6	2.5	3.2	2.7	0.1	—.2	0.2	0.1	0.2	—.1	L	+	0	+++
111	Tonsil, acute tonsillitis	?	1.8	2.5	2.0	2.4	0.1	0.0	0.3	0.2	0.2	0.1	L	0	0	++
8	Tonsil, acute tonsillitis	?	1.0	1.0	0.9	1.1	—.1	—.3	—.2	0.6	0.6	0.5	L	0	0	++
104	Throat, acute pharyngitis	?	1.8	1.4	2.2	2.0	0.4	0.0	0.1	0.0	0.0	—.2	B	P	0	+++
31	Sputum, acute pharyngitis	?	4.1	4.0	4.0	3.3	0.6	0.5	0.3	0.1	0.5	—.1	M	+	0	+
17	Throat, diphtheria	?	1.9	2.7	2.7	3.1	0.6	0.4	0.7	0.4	0.6	...	L	+	0	++
42	Sputum, pertussis	?	1.9	2.8	2.1	2.5	0.3	0.1	—.2	—.2	0.0	...	L	+	0	++
118	Sputum, bronchiectasis	?	1.9	1.9	2.1	1.0	0.2	—.2	0.1	0.0	—.1	0.6	LL	0	0	+
50	Sputum bronchitis	?	1.2	1.2	1.6	1.2	0.5	0.7	0.2	0.0	...	...	LL	0	0	++
12	Tonsil, chronic tonsillitis	?	2.3	2.5	2.6	2.6	0.2	0.0	—.1	0.2	0.2	0.5	B	0	0	+
23	Tonsil, chronic tonsillitis	?	2.4	3.4	2.8	2.6	0.3	0.4	—.1	0.1	0.2	...	L	+	..	++
66	Tonsil, chronic tonsillitis	?	1.7	1.9	2.2	0.9	0.0	0.0	—.2	2.4	0.0	...	B	0	0	0
114	Nose, sinusitis	?	1.8	2.2	2.8	2.2	0.2	0.3	0.0	0.4	0.3	0.2	L	+	0	++
27	Gum, pyorrhea	?	2.2	2.6	2.8	2.7	0.5	0.2	0.4	2.9	0.3	0.1	L	+	0	+
15	Air	S	2.6	0.9	1.2	1.8	0.1	—.1	0.3	0.4	0.3	0.3	..	+	0	....
109	Milk	S	6.1	4.7	5.2	3.9	0.4	0.1	0.1	0.3	0.1	.2	B	+	0	0

Serial No.	Source	Pathogenicity	Dextrose	Lactose	Saccharose	Salicin	Raffinose	Mannite	Inulin	Starch	Glycerin	Arabinose	Morphology	Milk	Neutral Red	Hemolysis
	GROUPS 2 AND 3, SALIVARY TYPES FERMENTING RAFFINOSE															
53	Pus, otitis media.....	P	3.1	2.5	1.7	—.2	2.1	—.2	0.4	—.1	—.1	...	L	+	0	++
123	Blood, endocarditis ...	P	2.5	2.6	2.8	0.7	3.9	.2	—.1	0.0	0.0	0.0	L	+	0	0
157	Blood, endocarditis ..	P	2.1	2.6	3.2	—.1	2.7	—.1	0.1	0.0	0.0	0.0	B	+	0	0
57	Sputum, asthma	?	2.0	2.3	1.3	—.2	1.9	—.5	0.4	0.2	0.3	...	M	+	0	+
81	Gum, pyorrhea	?	2.8	2.6	3.0	—.2	2.6	0.0	0.1	0.0	0.0	—.7	B	+	0	0
106	Tonsil, chronic tonsillitis	?	3.6	3.0	3.2	0.3	2.7	—.2	0.1	—.2	0.1	—.3	B	+	0	0
26	Tonsil, chronic tonsillitis	?	4.3	4.6	4.9	5.3	4 9	0.1	0.1	0.3	0.1	...	B	+	+	+
29	Gum, pyorrhea	?	4.1	4.0	4.2	3.9	3.8	0.0	0.1	0.1	0.1	0.0	B	+	0	+
51	Sputum, bronchitis	?	1.5	1.9	2.6	1.7	2.4	0.2	0.0	2.3	0.2	...	L	0	0	++
67	Tonsil, chronic tonsillitis	?	2.1	2.4	2.6	1.9	2.1	0.1	0.2	2.7	0.3	0.0	..	+	0	0
137	Tonsil, normal	S	4.5	3.7	3.8	0.6	4.7	0.1	0.2	0.1	0.1	—.2	M	+	0	0
138	Tonsil, normal.	S	4.8	4.5	5.2	5.0	4.0	0.0	0.0	0.2	—.1	—.2	L	+	0	0
140	Tonsil, normal	S	4.8	3.6	3.2	3.3	2.9	—.1	0.0	0.1	—.2	—.2	M	+	0	0
142	Tonsil, normal	S	5.4	2.9	5.1	3.7	3.0	0.0	0.4	0.2	0.0	0.0	B	+	0	+
	GROUP 4, SALIVARY TYPE FERMENTING INULIN															
144	Blood, endocarditis ...	P	3.3	2.0	3.4	3.4	0.1	—.3	3.3	0.0	—.1	0.0	B	+	0	0
130	Blood, endocarditis ...	P	4.5	4.1	4.3	4.1	3.9	2.8	2.1	2.0	—.3	—.2	..	+	0	0
127	Blood, endocarditis ...	P	4.2	4.2	3.7	3.7	3.0	1.6	2.5	0.0	0.0	0.3	M	+	0	0
68	Sputum, bronchitis ...	?	1.6	1.5	2.2	—.1	1.3	—.1	0.9	0.0	0.2	...	L	0	0	+
101	Sputum, bronchitis ...	?	3.0	3.1	3.6	3.5	2.1	—.2	1.9	.1	0.0	—.6	L	+	0	+
153	Gum, pyorrhea	?	5.4	5.8	5.6	5.0	0.5	0.1	2.3	0.3	0.1	0.7	..	+	0	0
96	Sputum, pneumonia ..	?	2.5	2.7	2.9	0.0	2.6	0.1	0.8	0.4	0.3	—.2	B	+	0	0
112	Tonsil, normal	S	2.9	3.8	3.8	2.9	0.3	0.2	3.3	0.2	0.2	0.2	B	+	0	++
113	Tonsil, normal	S	2.9	2.9	1.9	1.5	3.0	0.0	2.1	0.6	0.2	0.0	L	0	0	++
143	Tonsil, normal	S	3.1	2.6	2.6	—.1	2.8	0.2	2.3	0.5	0.0	—.1	M	+	0	+
141	Tonsil, normal	S	3.6	3.0	3.3	0.1	3.1	—.1	1.9	0.2	0.0	—.2	L	+	0	0
	GROUP 5, SALIVARY TYPE NOT FERMENTING SALICIN, RAFFINOSE, MANNITE OR INULIN															
13	Blood, sepsis meningitis	P	2.5	2.7	2.0	0.2	0.4	0.3	0.0	0.2	0.3	...	L	0	0	++
122	Blood, endocarditis....	P	2.7	1.8	2.4	0.0	0.1	—.2	0.0	—.1	0.0	0.2	L	+	0	0
125	Blood, endocarditis ...	P	2.8	2.9	3.0	0.1	0.0	—.5	—.4	—.3	—.3	0.1	..	+	0	0
129	Blood, endocarditis ...	P	3.1	2.2	2.7	—.1	0.5	0.0	—.2	0.3	0.1	0.4	B	+	0	+
131	Blood, endocarditis ...	P	3.1	3.0	3.9	—.2	0.4	—.1	—.1	0.0	—.2	0.0	L	+	0	0
151	Chancroid	?	2.3	2.5	2.2	—.2	0.2	0.0	0.0	0.7	0.1	0.3	..	..	..	0
11	Gum, pyorrhea	?	2.2	3.6	1.9	—.2	—.3	—.5	—.2	—.3	—.3	...	B	+	0	0
25	Gum, pyorrhea	?	1.6	1.8	1.9	0.5	0.4	0.3	0.2	...	...	...	LL	..	0	+
110	Tonsil, chronic tonsillitis	?	4.2	3.7	3.7	—.4	—.1	—.4	0.0	0.0	—.2	—.6	B	+	+	0
60	Eye, conjunctivitis	?	2.0	1.7	1.7	—.1	.1	—.1	0.0	...	0.0	...	L	0	0	0
115	Sputum, normal	S	3.4	3.1	2.8	—.2	0.1	—.1	—.2	0.0	—.1	—.1	B	+	0	0
43	Milk	S	2.0	1.9	2.4	0.1	0.3	0.3	0.3	2.1	0.3	...	L	+	0	+

Group 6, Fecal Type

20	Blood, sepsis puerperal	P	**2.3**	**1.7**	**2.3**	**3.1**	0.2	**3.0**	0.6	0.2	0.3	0.5	B	0	0	++
14	Pus, otitis media	P	**2.1**	**2.2**	**2.2**	**2.5**	0.2	**1.9**	0.1	0.1	0.2	...	B	0	0	++
128	Pus, otitis media	P	**1.9**	**2.0**	**2.0**	**1.3**	0.3	**1.8**	—.1	0.0	0.0	0.1	B	0	0	++
65	Pus, otitis media	P	**1.2**	0.0	**1.6**	0.0	0.4	**1.4**	0.1	0.3	0.1	...	B	0	0	....
74	Pus, abscess prostate..	P	**3.6**	**2.1**	**0.8**	**3.1**	0.1	**1.9**	0.1	0.4	0.4	0.2	M	0	0	0
133	Blood, endocarditis ..	P	**4.3**	**3.8**	**2.6**	**3.2**	**2.1**	**2.4**	0.1	0.4	0.1	...	B	+	0	0
75	Blood, endocarditis ...	P	**3.3**	0.1	**1.8**	**3.3**	0.0	**2.3**	0.1	**0.3**	0.5	0.2	B	P	0	0
107	Urine, cystitis	?	**5.6**	**4.3**	**1.1**	**2.8**	—.1	**2.4**	0.0	**1.8**	—.1	—.7	B	+	+	0
98	Stool	S	**3.8**	**3.1**	**3.7**	**4.0**	0.1	**2.5**	—.1	0.1	0.1	—.2	B	0	0	0
116	Stool	S	**3.6**	**3.8**	**2.7**	**4.2**	0.5	**3.2**	0.4	0.3	**1.0**	0.1	B	+	+	0
121	Stool	S	**4.7**	**3.8**	**3.2**	**4.2**	0.0	**3.1**	0.3	0.1	**0.8**	0.2	B	P	0	0
99	Milk	S	**3.4**	**2.6**	**2.3**	**3.8**	0.2	**2.4**	0.1	0.2	0.3	...	B	0	0	0
108	Milk	S	**5.0**	**4.0**	**4.4**	**2.4**	0.4	**2.8**	—.1	—.1	0.4	—.5	B	+	+	0

Group 7, Unclassified

1	Pus, abscess	P	2.3	0.4	0.8	—.2	—.1	0.2	—.1	—.1	—.1	—.1	M	0	0	++
154	Tonsil, chronic tonsillitis	?	3.0	0.6	2.2	2.9	0.5	0.1	0.3	1.9	0.6	0.7	L	0	0	++
132	"Strept. rheumaticus"..	P	4.8	2.5	—.1	4.2	—.1	—.2	—.4	0.0	—.1	—.1	B	+	0	0

Group 8, Pneumococcus

156	Neufeld II	P	**3.0**	**3.1**	**3.4**	0.3	**2.5**	—.2	**2.3**	0.1	0.4	0.1	B	+	0	+
100	Lung, pneumonia	P	**2.4**	**2.7**	**2.8**	0.3	0.3	0.1	**2.6**	0.3	0.7	0.3	B	0	0	0
120	Pus, urethritis	?	**2.6**	**2.6**	**2.5**	**2.6**	0.3	1.1	**2.3**	0.5	0.2	0.0	B	+	0	0
63	Blood, sepsis	P	**1.8**	**1.9**	**1.3**	0.0	0.0	0.1	0.1	—.1	—.2	...	B	+	0	0
62	Blood, sepsis, meningitis	P	**2.3**	**2.4**	0.1	—.1	0.0	0.2	0.0	—.1	—.2	...	M	0	0	0
105	Pus, empyema	P	**2.6**	**2.1**	0.0	0.1	0.4	0.2	0.2	0.0	0.3	0.6	B	+	+	0
117	Pus, empyema	P	**2.3**	**2.6**	—.3	—.5	0.2	—.5	0.6	—.4	—.4	0.2	B	0	0	0
149	Pus, empyema	P	**2.8**	**2.8**	—.1	0.0	0.0	—.2	—.2	0.1	—.1	.7	B	..	..	....

Group 9, Pneumococcus Mucosus

150	Pus, meningitis	P	**3.2**	**3.2**	**0.0**	**0.0**	—.2	—.1	—.1	0.1	0.1	...	B	0	0	+
134	.....	P	**2.3**	**2.4**	**2.3**	0.3	**2.2**	0.1	0.2	0.2	0.2	...	B	+	0	+
135	.....	P	**2.6**	**2.3**	**2.3**	0.2	**2.5**	—.1	**2.1**	0.1	0.1	0.1	B	+	0	0
136	.....	P	**2.5**	**2.3**	**1.8**	0.1	**2.5**	—.2	**2.3**	—.1	0.1	0.2	B	+	0	+

Positive reactions are indicated by black-face type.
Symbols: Under Pathogenicity: "P" = pathogenic; "?" = probably pathogenic; under Morphology; "B" = brevis; "M" = medius; "L" = longus; "LL" = longior. Under Milk; + = coagulated; 0 = uncoagulated; "P" = peptonized.

2. The tests are best performed in infusion broth colored with litmus, containing 2 per cent. or more of peptone and 1 per cent. of the fermentable substance. The initial reaction should be between 0.0 and 0.5 per cent. normal acidity (phenolphthalein). Only a complete change of the litmus to pink should be considered positive. The useful test substances are lactose, salicin, raffinose, mannite and inulin.

3. On the basis of these reactions we can recognize one pathogenic and six saprophytic groups:

(*a*) Pyogenic group; characterized by fermentation of lactose and salicin.

(*b*) Four salivary groups; two characterized by fermentation of raffinose, one by fermentation of inulin and one by failure to ferment salicin, raffinose, mannite or inulin.

(*c*) Fecal group; characterized by fermentation of mannite.

(*d*) Equine group; characterized by failure to ferment lactose.

We mention this last group, with which we have had no experience, for the sake of completeness. Its existence seems well established by the work of other investigators. The streptococci in this class are saprophytes and are commonly found in horse dung.

4. Titrations of the amount of acid formed in fermentation are of no value in classification.

5. Tests for ability to hemolyze human blood are of value in that highly pathogenic strains are usually strongly hemolytic.

6. The type of chain formation, the milk coagulation test, and the neutral red reaction, are not reliable as bases for classification.

These conclusions, being based on observation of only 105 strains, are necessarily tentative. The validity of the fermentation tests depends chiefly on whether or not they remain constant in a given race. Work along the lines opened up by Rosenow may show that we can artificially alter the fermentative powers of streptococci as well as their other properties. It is our purpose to preserve some of our strains for a year or more on artificial media to determine whether their fermentative powers change. We believe, however, that the reactions that we have described are characteristic of certain types of streptococci as they are isolated from human disease.